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The Class A Scavenger Receptor Binds to Proteoglycans and Mediates Adhesion of Macrophages to the Extracellular Matrix*

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The class A scavenger receptor (SR-A) binds modified lipoproteins and has been implicated in cholesterol ester deposition in macrophages. The SR-A also contributes to cellular adhesion. Using *SR-A*$^{+/+}$ and *SR-A*$^{-/-}$ murine macrophages, we found SR-A expression important for both divalent cation-dependent and -independent adhesion of macrophages to the human smooth muscle cell extracellular matrix. The SR-A mediated 65 and 85% of macrophage adhesion to the extracellular matrix in the presence and absence of serum, respectively. When EDTA was added to chelate divalent cations, the SR-A mediated 90 and 95% of the macrophage adhesion without and with serum, respectively. SR-A-mediated adhesion to the extracellular matrix was prevented by fucoidin, an SR-A antagonist. Biglycan and decorin, proteoglycans of the extracellular matrix, were identified as SR-A ligands. Compared with control cells, Chinese hamster ovary cells expressing the SR-A showed 5- and 6-fold greater cell association (binding and internalization) of ^{125}I-decorin and -biglycan, respectively. In competition studies, unlabeled proteoglycan or fucoidin competed for binding of ^{125}I-labeled decorin and -biglycan, and biglycan and decorin competed for the SR-A-mediated cell association and degradation of ^{125}I-labeled acetylated LDL, a well characterized ligand for the SR-A. These results suggest that the SR-A could contribute to the adhesion of macrophages to the extracellular matrix of atherosclerotic plaques.

Macrophages express several scavenger receptors that bind modified lipoproteins (1–5). The cloning and identification of individual scavenger receptors have permitted a detailed assessment of their functions. The type I and type II class A scavenger receptors (SR-A)[1] were the first to be cloned (6, 7). These receptors are trimeric integral membrane glycoproteins generated by alternative splicing of a single gene product (1, 6, 7). They both bind a wide variety of molecules, including certain chemically modified lipoproteins such as oxidized low density lipoproteins (LDL) and acetylated LDL (AcLDL), certain polysaccharides such as fucoidin and dextran sulfate, and polyribonucleotides such as poly I and poly G (1). In addition, the SR-A binds a limited number of native proteins. We recently showed that the α-secretase cleavage products of the three main isoforms of the amyloid precursor protein (APP695, APP751, and APP770) are ligands for the SR-A (8). The SR-A also binds the amyloid-β (Aβ) peptide (9), another enzymatic cleavage product of APP and a major component of senile plaques in Alzheimer's disease (AD).

Macrophage scavenger receptors have been postulated to contribute to the internalization of modified lipoproteins, intracellular cholesterol ester accumulation, foam cell formation, and atherogenesis (10). Numerous studies have suggested that the SR-A is critically involved in the deposition of cholesterol in arterial wall macrophages during the development of atherosclerotic lesions. Elimination of SR-A expression in mice significantly reduces the uptake of modified LDL by peritoneal macrophages from these animals and inhibits atherogenesis (11–13). Although reduced atherosclerosis in *SR-A*$^{-/-}$ mice is widely assumed to result from reduced uptake of modified lipoproteins and reduced accumulation of cholesterol esters in macrophages of the arterial wall, this mechanism has not been proved experimentally. SR-A may, in fact, contribute to atherogenesis in ways distinct from the function of SR-A in the uptake of modified lipoproteins (14). The SR-A also may play an important role in immune response and in cell adhesion (11, 15–20). In fact, it has been suggested that the SR-A may interact with components of the subendothelial space, thereby contributing to the adhesion and retention of macrophages in the artery wall (11, 15–19).

To examine this possibility further, we tested the hypothesis that the SR-A contributes to the adhesion of cells to the extracellular matrix. We found that biglycan and decorin, components of the extracellular matrix, as well as aggrecan, a proteoglycan that is similar to versican (a prominent matrix component) are ligands of the SR-A and that the SR-A contributes significantly to the divalent cation-dependent and -independent adhesion of macrophages to extracellular matrix derived from both smooth muscle cells and endothelial cells.

EXPERIMENTAL PROCEDURES

Materials—Bovine aggrecan, bovine biglycan, bovine decorin, hyaluronan, and fucoidin were obtained from Sigma. Collagen IV was obtained from ICN Biomedicals (Costa Mesa, CA). PD-10 columns and Na^{125}I were purchased from Amersham Biosciences. Dulbecco's modified Eagle's medium, F12 medium, RPMI 1640 medium, trypsin-EDTA solution, and penicillin-streptomycin solution were purchased from Invitrogen. Fetal bovine serum was obtained from Hyclone (Logan, UT). Tissue culture dishes and other plastic ware were obtained from Falcon

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[1] The abbreviations used are: SR-A, class A scavenger receptor(s); LDL, low density lipoprotein(s); AcLDL, acetylated LDL; Aβ, amyloid β; AD, Alzheimer's disease; CHO, Chinese hamster ovary; HSMC, human smooth muscle cell; PBS, phosphate buffered saline; APP, amyloid precursor protein(s).

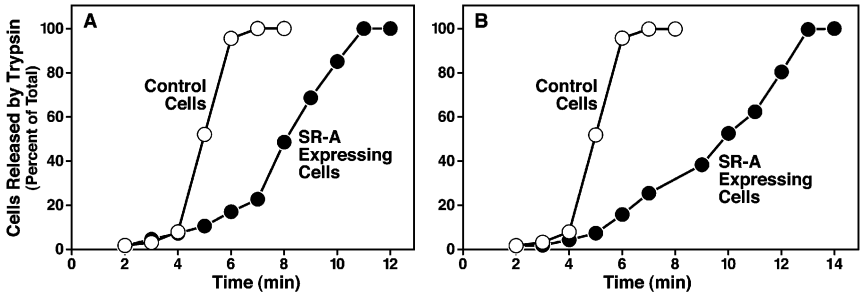

FIG. 1. **Adherent CHO cells expressing SR-A are more resistant to release by trypsin than control CHO cells.** Control CHO cells and CHO cells expressing the SR-A were grown to confluence on tissue culture plates (*A*) or on plates coated with extracellular matrix elaborated by HSMC (*B*). The cells were washed with PBS and treated with trypsin-EDTA for the indicated times at room temperature. Trypsin activity was stopped by the addition of serum-containing medium, and the cells that were released into the medium were quantitated. The data are the average of two independent experiments in a series of four with similar results.

(Franklin Lakes, NJ), Corning (Acton, MA), and Nalge Nunc International (Rochester, NY).

Lipoproteins and Proteoglycans—Human LDL (d = 1.02–1.05 g/ml) were isolated from the plasma of normal fasted donors by sequential ultracentrifugation at 4 °C (21). The LDL were radiolabeled by the iodine monochloride method (22) to a specific activity of 150–350 cpm/ng of protein. AcLDL was prepared by treating LDL with acetic anhydride (23). Lipoproteins were dialyzed against 0.15 M NaCl and 0.01% EDTA, pH 7.2, before use. Aggrecan, biglycan, and decorin were labeled with ^{125}I using iodobeads (Pierce) as recommended by the manufacturer. The specific activities were 200–400 cpm/ng.

Cell Release Assay—Control Chinese hamster ovary (CHO) cells were grown in 95% air/5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium/F12 medium containing 10% fetal bovine serum. CHO cells stably transfected to express the type I SR-A were grown in the same medium containing 400 μg/ml of G418. The preparation and properties of CHO cells stably expressing the SR-A have been described previously (24). The cells were grown to confluence either in untreated 35- or 60-mm tissue culture plates or in plates coated with the extracellular matrix from human smooth muscle cells (HSMCs). The extracellular matrix was prepared as described below. The plates were washed three times with phosphate buffered saline (PBS) and incubated with 1 ml of trypsin-EDTA solution at room temperature on an orbital platform. Trypsin activity was stopped at the times indicated with 1 ml of serum-containing medium. An aliquot of the medium was taken, and the number of cells released was determined with a cell counter (Coulter).

Cell Adhesion Assays—HSMCs and bovine aortic endothelial cells were grown to confluence in 96-well plates, and the extracellular matrix was prepared as described (25). Briefly, the cells were washed three times with PBS and incubated with 0.5% Triton X-100 in H_2O for 10 min at room temperature. The wells were then washed three times with PBS and dried. Thioglycollate-elicited peritoneal macrophages from *SR-A*$^{+/+}$ or *SR-A*$^{-/-}$ mice (11) were obtained by peritoneal lavage, concentrated by centrifugation, resuspended in RPMI 1640 medium, and counted with a cell counter (Coulter). Macrophages (3×10^4) were seeded in wells coated with extracellular matrix, incubated for 1.5 h at 37 °C under the conditions indicated, and washed three times with PBS. Adherent cells were fixed with 3% paraformaldehyde in PBS and their nuclei stained with Sitox green (Molecular Probes, Eugene, OR) in PBS. Fluorescence images were captured with a Nikon Eclipse TE 300 inverted microscope equipped with a digital camera (Diagnostic Instruments SPOT RT, Sterling Heights, MI). Image processing was used to isolate the nuclei from background fluorescence produced by the extracellular matrix, and then the nuclei in each image (a standard field captured using a ×10 objective) were counted. Five or six images were captured for each condition from triplicate wells of a 96-well plate. The images were processed and quantitated with FoveaPro Version 1.0 (Reindeer Graphics, Asheville, NC), running with PhotoShop 6.0 (Adobe, San Jose, CA) on a Macintosh G4 computer.

Cell Association and Degradation of Proteoglycans and Lipoproteins—Control CHO cells and CHO cells expressing the SR-A were grown in 12-well plates as indicated above, washed three times with serum-free medium, and incubated for 5 h at 37 °C in serum-free medium containing ^{125}I-aggrecan, -biglycan, or -decorin (1 μg/ml) alone or with the indicated competitors. Experiments were also performed with ^{125}I-AcLDL (2 μg/ml) alone or with the indicated concentrations of competitors. Cell-associated ligands (*i.e.* bound and internalized protein) and degraded ligands (trichloroacetic acid-soluble protein degradation products in the medium) were quantitated as described (26, 27).

RESULTS

SR-A Expression Contributes to Trypsin Resistance—We observed that SR-A-expressing cells were more resistant to trypsin release than control cells. To quantitate this effect, cells were grown to confluence in 35- or 60-mm plates, washed, treated with trypsin/EDTA solution for different periods of time, and counted. CHO cells expressing the SR-A were more resistant to trypsin release than control CHO cells, which do not express the SR-A. Starting at 5 min of incubation with trypsin, a striking difference in trypsin release of the SR-A-expressing and nonexpressing cells was evident (Fig. 1). All control cells were released at 6 min of incubation with trypsin/EDTA, whereas it took 11–12 min for the release of all of the SR-A expressing cells. This difference was even greater when the experiment was performed with cells grown on plates coated with extracellular matrix from HSMCs (Fig. 1*B*). Expression of the SR-A therefore contributes to adhesion of the cells either to the cell culture dishes or to the matrix elaborated by the cells.

SR-A Mediates Macrophage Adhesion to the Extracellular Matrix—We examined thioglycollate-elicited macrophages from *SR-A*$^{+/+}$ and *SR-A*$^{-/-}$ mice for their ability to adhere to the extracellular matrix produced by HSMCs. Under all conditions tested, macrophages from *SR-A*$^{-/-}$ mice were less effective in adhering to the coated wells than macrophages from *SR-A*$^{+/+}$ mice (Fig. 2). In the absence of serum, the adhesion of *SR-A*$^{-/-}$ macrophages to the HSMC extracellular matrix was 10% of the adhesion of *SR-A*$^{+/+}$ macrophages in the presence of EDTA (to chelate divalent cations) and 16% in the absence of EDTA. In the presence of serum, the adhesion of *SR-A*$^{-/-}$ macrophages to the HSMC extracellular matrix was ~35% of the adhesion of *SR-A*$^{+/+}$ macrophages in the absence of EDTA and ~5% in the presence of EDTA (Fig. 2). Similar results were obtained when experiments were performed with an extracellular matrix from bovine aortic endothelial cells (data not shown). The SR-A antagonist fucoidin inhibited 70–90% of the adhesion of the *SR-A*$^{+/+}$ macrophages (Fig. 2). Taken together these results demonstrate that the SR-A contributes significantly to the divalent cation-dependent and -independent adhesion of thioglycollate-elicited macrophages to extracellular matrix, both in the presence and absence of serum.

Aggrecan, Biglycan, and Decorin Are Ligands for the SR-A—We next identified two components of extracellular matrix that are SR-A ligands. Soluble biglycan and decorin were radioactively labeled and tested for their ability to bind to the SR-A. Compared with control cells, CHO cells stably expressing the SR-A showed a 5- to 6-fold greater cell association (which represents bound and internalized ligand) of ^{125}I-labeled decorin and biglycan (Fig. 3). We also found that aggrecan, a proteoglycan found in cartilage that is similar to the extracel-

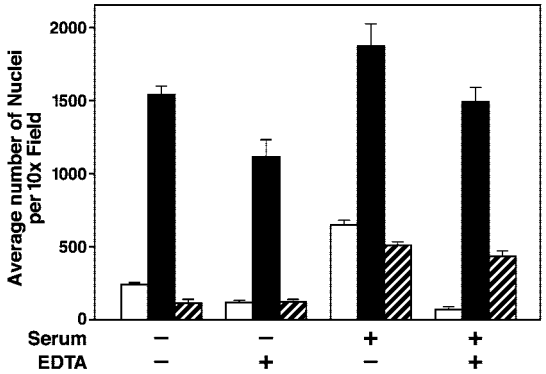

FIG. 2. **The SR-A mediates macrophage adhesion to the extracellular matrix.** Macrophages from *SR-A*$^{-/-}$ (*white bars*) and *SR-A*$^{+/+}$ (*black bars*) mice were seeded in 96-well plates coated with extracellular matrix from HSMC, incubated for 1.5 h at 37 °C with or without serum and EDTA as indicated, washed with PBS, and fixed with 3% paraformaldehyde. Nuclei of adherent cells were counted after Sitox green staining. Adhesion of *SR-A*$^{+/+}$ macrophages was also quantitated in the presence of 100 μg/ml fucoidin (*hatched bars*). Results are the mean ± S.D. of five independent fields from triplicate wells of a representative experiment, in a series of three with similar results.

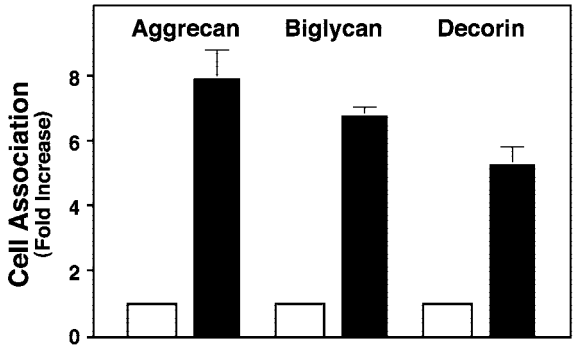

FIG. 3. **Aggrecan, biglycan, and decorin bind to the SR-A.** Control CHO cells (*white bars*) and CHO cells expressing the SR-A (*black bars*) were incubated for 5 h at 37 °C with ^{125}I-aggrecan, ^{125}I-biglycan, or ^{125}I-decorin (1 μg/ml). The cells were washed and the cell association of labeled proteoglycans was determined. The results are expressed as the -fold increase in cell association after subtraction of the nonspecific binding obtained in the presence of 10 μg/ml fucoidin. Results are the mean ± S.D. of three independent experiments performed in duplicate.

lular matrix component versican, is a ligand for the SR-A. SR-A-expressing cells showed an ~8-fold greater cell association of ^{125}I-labeled aggrecan than control cells (Fig. 3).

To examine further the specificity of interaction of the proteoglycans with the SR-A, the cell association and degradation of aggrecan, biglycan, and decorin were tested in competition experiments with unlabeled proteoglycan and fucoidin (Fig. 4). A 10-fold molar excess of aggrecan or fucoidin competed for 80–90% of the cell association and 80–85% of the degradation of ^{125}I-aggrecan. Biglycan or fucoidin (10-fold molar excess) also competed for 80–90% of the cell association and ~80% of the degradation of ^{125}I-biglycan, whereas a 10-fold excess of decorin or fucoidin competed for only 50–60% of the cell association and ~50% of the degradation of ^{125}I-decorin. Higher concentrations (as high as a 100-fold excess) of competitor did not result in a substantially higher competition.

Aggrecan, biglycan, and decorin also competed for the cell association and degradation of ^{125}I-AcLDL by CHO cells expressing the SR-A (Fig. 5). Aggrecan, a high molecular weight proteoglycan, was the most effective competitor for the SR-A-mediated cell association and degradation of AcLDL, whereas biglycan was as effective as AcLDL and fucoidin (Fig. 5). Decorin was a less potent competitor.

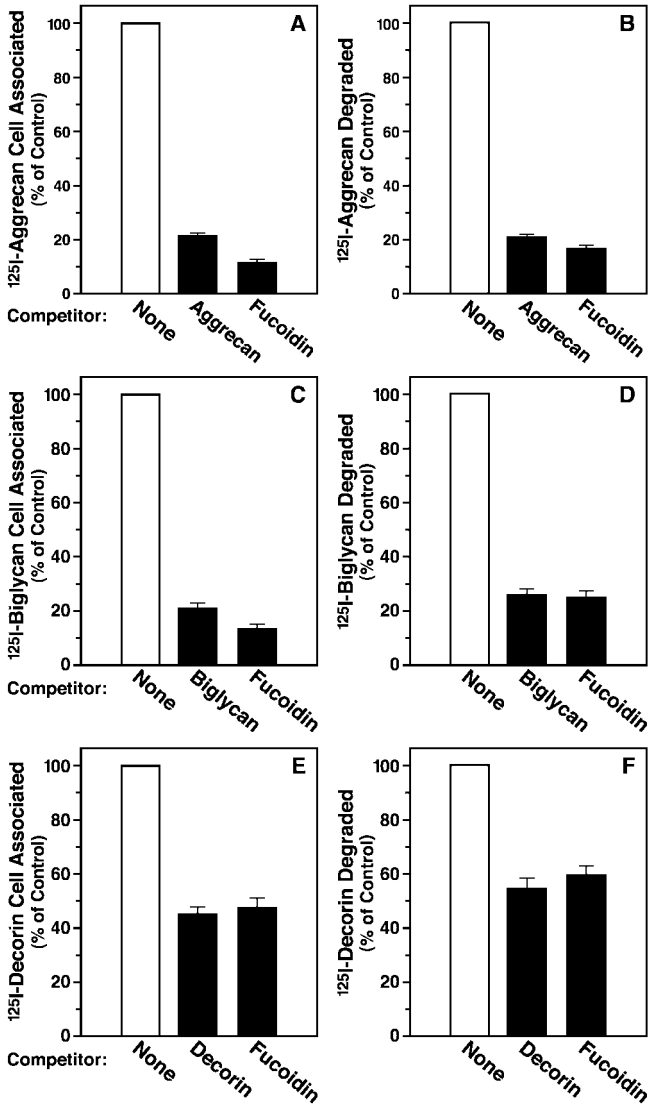

FIG. 4. **Fucoidin and nonlabeled proteoglycans compete for the cell association and degradation of ^{125}I-aggrecan, ^{125}I-biglycan, or ^{125}I-decorin by CHO cells expressing the SR-A.** CHO cells expressing the SR-A were incubated for 5 h at 37 °C with ^{125}I-aggrecan (*A*, *B*), ^{125}I-biglycan (*C*, *D*), or ^{125}I-decorin (*E*, *F*) (1 μg/ml) alone (*white bars*) or in the presence of unlabeled competitor (10 μg/ml) (*black bars*). The cells were washed and the cell association or degradation of labeled proteoglycans was determined. Results are the mean ± S.D. of three independent experiments performed in duplicate.

We also examined the ability of hyaluronan and collagen IV, other components of extracellular matrix, to compete for the SR-A-mediated cell association and degradation of AcLDL. We found that hyaluronan and collagen IV did not compete with AcLDL even at concentrations of 100 μg/ml (data not shown).

DISCUSSION

This study shows that the SR-A contributes to the divalent cation-dependent and -independent adhesion of macrophages to extracellular matrix from HSMC and endothelial cells in the presence and absence of serum. This adhesion was inhibited by the SR-A antagonist fucoidin. Expression of SR-A by CHO cells delayed the release of cells from tissue culture dishes and from HSMC extracellular matrix by trypsin. Furthermore, we identified soluble biglycan and decorin, proteoglycans of the extracellular matrix, as ligands for the SR-A. In competition experiments, biglycan competed as effectively as AcLDL and fucoidin for the SR-A-mediated cell association and degradation of ^{125}I-

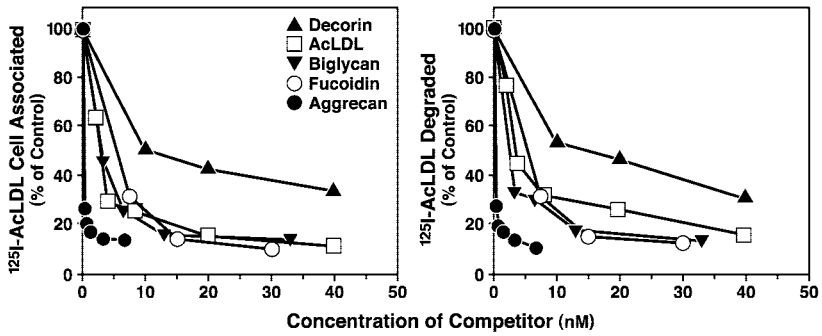

FIG. 5. **Aggrecan, biglycan, and decorin compete for the cell association and degradation of ^{125}I-AcLDL by CHO cells expressing the SR-A.** The cells were incubated for 5 h at 37 °C with ^{125}I-AcLDL (2 μg/ml) alone or with increasing concentrations of competitors. The cell association (*left panel*) and degradation (*right panel*) of ^{125}I-AcLDL were determined. In the absence of competitors, the cell association and degradation of ^{125}I-AcLDL were 192 ± 9 and 216 ± 13 ng/mg cell protein, respectively. Each data point represents the mean ± S.D. of three independent experiments performed in duplicate. The experimental *error bars* are within the data points.

AcLDL. Decorin was less effective, suggesting that it has a lower affinity for the SR-A than biglycan or AcLDL. Unlabeled decorin or fucoidin competed only partially for binding of ^{125}I-decorin to CHO cells expressing the SR-A. The binding of versican, another prominent component of the extracellular matrix, was not examined directly. However, we determined that aggrecan, a predominant proteoglycan in cartilage that is analogous to versican (28, 29), is a ligand for the SR-A and a better competitor for the cell association and degradation of AcLDL than either biglycan or decorin. In contrast to biglycan and decorin, the core proteins of both aggrecan and versican are extensively substituted with chondroitin sulfate (28).

Macrophages exhibit both Ca^{2+}-dependent and -independent adhesion. Unlike other cell types, macrophages attach to tissue culture plastic in the absence of divalent cations. In the presence of EDTA, which chelates Ca^{2+} and Mg^{2+}, macrophages lose their spread morphology but remain adherent. The first indication that the SR-A contributes to adhesion was the observation that a monoclonal antibody to the SR-A (2F8) totally inhibits the divalent cation-independent adhesion of murine macrophages to tissue culture plastic in the presence but not in the absence of serum, suggesting that a component of serum is necessary for this adhesion (15, 16). Studies using *SR-A*$^{-/-}$ and *SR-A*$^{+/+}$ macrophages showed that the SR-A mediates 50–60% of the adhesion of macrophages to tissue culture plastic in the presence of serum (11, 19). In the presence of EDTA to chelate divalent cations, more than 85% of the adhesion is SR-A dependent (11, 19).

The contribution of the SR-A to cell adhesion is dependent on the cell type and the cellular activation state. In resident peritoneal macrophages (not elicited) or resting Kupffer cells, cell adhesion does not differ in *SR-A*$^{+/+}$ and *SR-A*$^{-/-}$ cells, whereas in thioglycollate-elicited macrophages or phorbol ester-activated Kupffer cells, the SR-A is responsible for about 85 and 35%, respectively, of cell adhesion in the presence of serum (19). The SR-A also mediates the adhesion of macrophages and SR-A-transfected cells to glycated collagen type IV in the absence of serum and divalent cations (17), to activated β lymphocytes (20), and to sections of several mouse tissues (16, 18). The tissue components with which the SR-A interacts were not identified.

Our data show that the SR-A contributes to both the cation-dependent and -independent adhesion of thioglycollate-elicited peritoneal macrophages to the extracellular matrix from HSMCs, in either the presence or absence of serum. In the presence of serum, *SR-A*$^{-/-}$ macrophages showed 65 and 95% reduced adhesion (in the presence and absence of divalent cations, respectively) to extracellular matrix, compared with the adhesion of *SR-A*$^{+/+}$ macrophages. This difference in adhesion is similar to data reported previously using tissue culture plastic (11, 19). However, in the absence of serum the difference in adhesion to the extracellular matrix between *SR-A*$^{-/-}$ and *SR-A*$^{+/+}$ macrophages was much greater than the effect when adhesion to plastic was studied. *SR-A*$^{-/-}$ macrophages showed 84 and 90% decreased adhesion to the HSMC extracellular matrix (in the presence and absence of divalent cations, respectively) compared with the decreases in adhesion to tissue culture plastic of 28 and 29% (19). These data demonstrate that the interaction of the SR-A with components in the extracellular matrix contributes to the adhesion of thioglycollate-elicited macrophages and that this interaction is independent of serum and divalent cations.

We found that expression of the SR-A doubled the amount of time necessary to release cells with trypsin from tissue culture plates or tissue culture plates coated with extracellular matrix from HSMC. Our studies complement and extend previous studies using a different paradigm which suggested that the SR-A is partially responsible for the trypsin-resistant adhesion of macrophages to tissue culture plastic (16). In those studies, RAW 264 macrophages were gently trypsinized and washed in serum-containing medium before adhesion assays. Trypsin release after adhesion was not studied. Under those conditions, the SR-A was determined to account for 15–20% of the trypsin-resistant cell adhesion (16).

The contribution of the SR-A to the adhesion of macrophages *in vivo* has not been demonstrated. However, our data and those reported previously suggest a role for the SR-A in adhesion under both normal and pathological conditions in both the vasculature and the central nervous system. The SR-A is highly expressed on activated microglia in the vicinity of Aβ-containing senile plaques in brains of patients with AD (30). The SR-A binds and internalizes microaggregates of the 42-amino acid form of Aβ *in vitro* (9). In addition, we recently reported that the SR-A binds secreted forms of APP (8). The Aβ peptide and secreted APP are major constituents of senile plaques and cerebrovascular deposits in patients with AD and Down's syndrome (31–33). The SR-A may contribute to the clearance of both Aβ and secreted APP, which are produced continuously in normal and AD brains (32–35). However, the SR-A also mediates the adhesion of microglia and human monocytes to β-amyloid fibril-coated surfaces, leading to secretion of reactive oxygen species and cell immobilization (36). The SR-A may, therefore, contribute to the adhesion of cells to the senile plaques and/or to cells expressing APP on their plasma membrane. In addition to Aβ and sAPP, AD plaques contain a wide variety of molecules, including extracellular matrix proteoglycans. It would be interesting to determine whether these molecules have a role in the interaction with microglia and in the progression of the disease.

The SR-A may also contribute to the adhesion of macrophages in atherosclerotic lesions. Expression of the SR-A contributes to the development of atherosclerosis. *SR-A*$^{-/-}$ mice had smaller atherosclerotic lesions than control mice (11–13). Because *SR-A*$^{-/-}$ macrophages exhibit a reduced uptake and degradation of modified LDL compared with wild-type macrophages (11, 37), it is widely assumed that a decrease in lipid accumulation in macrophages in the arterial wall is responsible for the reduced atherosclerosis. However, this has not been proved. Other aspects of SR-A biology have been suggested to contribute to the reduced atherosclerosis (11, 15–19). Our results support a potential alternative mechanism for the decrease in atherosclerosis in *SR-A*$^{-/-}$ mice. These data suggest that the adhesion and retention of *SR-A*$^{-/-}$ macrophages in the extracellular matrix may be lower than the retention of *SR-A*$^{+/+}$ macrophages, thereby resulting in reduced development of atherosclerosis. Biglycan, versican, and decorin, normally present in arteries, are substantially elevated in atherosclerotic lesions (38–40). In atherosclerotic plaques, decorin colocalizes with the macrophage-rich core (41, 42), whereas biglycan and versican are prominent in the smooth muscle cell matrix adjacent to macrophages (41, 43). Our data suggest that the interaction of the SR-A with these proteoglycans or other components of the extracellular matrix may contribute to the adhesion and retention of macrophages in atherosclerotic lesions, thereby enhancing lipid accumulation and the progression of the disease.

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